ANALYSIS OF THIODIGLYCOL IN WATER BY SINGLE REACTION MONITORING LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

SOP for Analysis in Environmental Water
MS015 Revision 0

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CRL METHOD MS015

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TABLE OF CONTENTS

DISCLAIMER

The mention of trade names or commercial products in this document is for illustrative purposes, and does not constitute an endorsement or recommendation for use by the U. S. Environmental Protection Agency.

1. Scope and Application

1.1 This procedure covers the determination of thiodiglycol that is analyzed by direct injection without derivatization by liquid chromatography/tandem mass spectrometry (LC/MS/MS). This compound is qualitatively and quantitatively determined by this method.

1.2 This is the Chicago Regional Laboratory (CRL) method for the analysis of thiodiglycol in water samples by LC/MS/MS utilizing the Waters 2695™ LC system and a Quattro micro™ mass spectrometer.

1.3 The limit of detection (LOD) and the CRL reporting limit (RL) for this compound are listed in Table 1. This standard operating procedure (SOP) has been tested on laboratory water and Chicago River water samples. The precision and accuracy (P&A) quality control acceptance criteria are shown in Table 2. Limit of Detection and P&A values will be updated regularly as more data is collected.

1.4 The statistical method, utilizing the Laboratory Control Standards (LCS) and their duplicates, will be used to calculate uncertainty as more data is collected. Uncertainty values will be provided after further inter-laboratory method validation.

1.5 Limit of detection limit (LOD) with precision and accuracy studies must be performed before an analytical SOP may be used and will be repeated for any major SOP revisions. These studies evaluate whether the reporting limits and calibration standard concentrations are appropriate. LOD studies must be performed annually for this method if values are reported below the reporting limit.

2. List of Acronyms and Abbreviations

CAS	Chemical Abstract Service
CCC	Continuing Calibration Check
CD	Compact Disc
CRL	Chicago Regional Laboratory
EPA	U.S. Environmental Protection Agency
IC	Initial Calibration
LC	Liquid Chromatography
LCS	Laboratory Control Sample
LOD	Limit of Detection
LV	Calibration Level
MDL	Method Detection Limit
MRM	Multiple Reaction Monitoring
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
MS/MSD	Matrix Spike/Matrix Spike Duplicate
MSP	Method Specific Parameter
NIST	National Institute of Standards and Technology
NPDES	National Pollution Discharge Elimination System
PPB	Parts per Billion
PPM	Parts per Million
PPT	Parts per Trillion
P&A	Precision and Accuracy
QA	Quality Assurance
QC	Quality Control
REC	Percent Recovery
RL	Reporting Limit
RLIMS	Relational Laboratory Information Management System
RSD	Relative Standard Deviation
RT	Retention Time
RTS	Retention Time Shift
SOP	Standard Operating Procedure
SRM	Single Reaction Monitoring
SS	Surrogate Standard
TC	Target Compound
TCL	Target Compound List

3. Summary of Method

3.1 For thiodiglycol analysis samples are shipped to the lab at 4°C. In the lab the samples are spiked with surrogates, filtered using a syringe driven Millex® HV PVDF filter unit and analyzed directly by LC/MS/MS within 7 days.

3.2 The target compound is identified by comparing the sample primary single reaction monitoring (SRM) transition to the known standard SRM transition. The retention time for the analytes of interest must also fall within the retention time of the standard by ±5%. The target compound is quantitated using the primary SRM transition utilizing an external calibration. The final report issued for each sample lists the total concentration of thiodiglycol, if detected, or the reporting limit, if not detected, in µg/L for water samples. Concentrations below the reporting limit will not be reported.

4. Health and Safety

The toxicity and carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound is treated as a health hazard. From this viewpoint, exposure to these chemicals is reduced to the lowest possible level. The laboratory maintains a web reference of material data safety sheets regarding the safe handling of the chemicals specified in this method. The address is www.msdsvault.com. The username in CRLUSER and password is CRLMSDS.

5. Cautions and Interferences

Method interferences may be caused by contaminants in solvents, reagents, glassware and other apparatus that lead to discrete artifacts or elevated baseline in the selected ion current profiles. All of these materials are routinely demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as the samples.

5.1 All reagents and solvents should be of pesticide residue purity or higher to minimize interference problems.

5.2 Matrix interferences may be caused by contaminants from the sample, sampling devices or storage containers. The extent of matrix interferences will vary considerably from sample source to sample source, depending upon variations of the sample matrix.

6. Apparatus and Materials

6.1. LC/MS System

6.1.1 Liquid Chromatograph (LC) System - An analytical system complete with a temperature programmable liquid chromatograph with a solvent mixer (Waters - Model 2695™) and all required accessories including syringes, solvent degasser and, autosampler. The injection port must be designed for 100 µL injection.

6.1.2 Analytical column - SIELC – Primesep SB™ 5 µm, 100 Å particle, 150 mm x 2.1 mm or equivalent.

6.1.3 Mass Spectrometer (MS) System – A Waters Quattro micro™ mass spectrometer was used. A mass spectrometer capable of MRM analysis with the capability to scan fast enough to obtain at least 14 scans over a peak with adequate sensitivity is required. Refer to mass spectrometer specifications for more detail.

6.1.4 Data Backup Device - A data archival unit (IBM computer; Microsoft Windows XP and Novell Netware file server- R5CRL) to archive data. All the lab generated data are also stored on the primary server. In addition, the laboratory has capabilities to store and retrieve data using other devices such as CD or DVD writers.

6.1.5 Data System - Masslynx™ Service Pack 4 or more recent must be interfaced to the LC/MS that allows the continuous acquisition and storage on machine-readable media of all mass spectra obtained throughout the duration of the chromatographic program. Quanlynx™ is used for all quantitation from data generated from the LC/MS.

6.1.6 Ultra pure nitrogen gas generator or equivalent nitrogen gas supply.

6.2. Glassware and Miscellaneous Supplies

6.2.1 Vials - 2-mL autosampler vials with teflon-lined screw tops.

6.2.2 Syringes - 0. 25 mL, 0.5 mL, 1 mL and 5.0 mL ±1% accuracy.

6.2.3 Micro-syringes - 100 µL, 50 µL, 25 µL and 10 µL ±1% accuracy.

6.2.4 Ultra pure Argon gas.

6.2.5 Analytical balance accurate to 0.1 mg; reference weights traceable to Class S or S-1 weights.

6.2.6 NIST traceable thermometer.

6.2.7 Class A volumetric glassware.

6.2.8 Grab sample bottle – 250 mL or larger amber glass, fitted with Teflon lined screw cap. If amber bottles are not available, the samples should be protected from light.

6.2.9 Millex® HV Syringe driven filter unit PVDF 0.45 µm (Millipore Corporation, Catalog # SLHV0033NS)

6.3. Reagents and Standards

When compound purity is assayed to be 98% or greater, the weight may be used without correction to calculate the concentration of the stock standard. All weights and concentrations listed in this SOP are corrected to 100% if less then 98% purity. Expiration time of the stock standards and all subsequent solutions is 7 days from the time prepared if stored protected from light (amber vials)

and at 4°C or less. The spiking standards and surrogates can be used longer than 7 days if they fall within +/- twenty percent of the given concentration using a new calibration curve were the standards are less than 7 days old and stored protected from light (amber vials) at 4°C or less.

6.3.1 Solvents – Acetonitrile, Methanol, Isopropyl Alcohol, and Water, HPLC mass spectrometry pesticide quality or equivalent.

6.3.2 Ammonium Formate (ACS Reagent Grade or better)

6.3.3 Formic Acid (Concentrated, ACS Reagent Grade or better)

6.3.4 Target Analyte- Thiodiglycol (CAS # 111-48-8)

6.3.5 Surrogate – 3,3'-Thiodipropanol (CAS # 10595-09-2)

6.3.6 Label all standards with LIMS ID; and verify the correct grade of solvents in comment field.

6.3.7 Traceability of standards is done using the manufacturer's specifications provided at time of purchase.

6.3.8 Verification procedures are not available against reference materials for this draft method at this time.

7. Instrument Calibration and Standardization Procedures

All calibration standard preparations should be noted in the LC/MS Standards Logbook and LIMS. All standard stock vials include the identification of target compounds, concentrations, expiration date and are referenced in LC/MS logbook. All small vials where space is an issue shall include a LIMS number which can be referenced back to the logbook. All stock solutions are prepared with methanol unless otherwise stated.

7.1 Surrogate Spiking Solution

Surrogate standard solution consisting of 3,3'-Thiodipropanol (CAS # 10595-09-2) is **added to all samples. The surrogate, 3,3'-thiodipropanol, is added to each sample in** order to achieve a concentration of 2.5 mg/L (i.e., 25 µL of a 2,500 ppm methanol solution containing 3,3'-thiodipropanol is added to a 25 mL water sample.)

7.2 Target Compound Spiking Solution

Each matrix spike or LCS/LCSD sample is spiked with thiodiglycol (CAS # 111-48-8) to achieve a concentration of 2.5 mg/L. (i.e., 25 µL of a 2,500 ppm methanol solution containing thiodiglycol is added to each 25 mL water quality control sample.)

7.3 Calibration Standards

Calibration stock standard solution is prepared from standard materials or purchased as certified solutions. A stock standard Solution A (Level 7) containing thiodiglycol and 3,3'-thiodipropanol in water was diluted to prepare Levels 1 through 6 as shown in Tables 3 & 4. All concentrated stock standard solutions are made in methanol. The resulting calibration standards made from these stock standards shall be prepared in water.

7.4 Calibration of Mass Spectrometer

The Waters Quattro micro™ mass spectrometer is calibrated monthly or when mass shifts of more than 0.5 Dalton are noticed by the analyst. The calibration file is saved in the Masslynx™ file folder. The calibration solution normally used is a mixture of NaCsI. Other calibration solutions can also be used per manufacturers specifications. The detailed procedure for calibrating the mass spectrometer can be found in the Masslynx™ instruction manual located near the instrument.

7.5 Quantitation of Target Analytes

The quantitation of the target analytes is accomplished with Quanlynx™ software. No internal standards are used. An external calibration is used along with monitoring 3,3'-thiodipropanol surrogate recovery. Refer to Table 5 for the SRM transitions and retention times.

7.6 Initial Calibration

The initial calibration contains a 7 point curve as shown in Tables 3 and 4. Depending on instrument type the sensitivity and calibration curve responses may vary. At a minimum, a five point linear or a six point quadratic calibration curve will be utilized for all analytes. The coefficient of the determination (r^2) of the linear fit must be greater than or equal to 0.98. The coefficient of the determination (r^2) of the quadratic curve must be greater than or equal to 0.99. A calibration curve and an instrument blank will be analyzed at the beginning of each run or daily to insure instrument stability. A new curve will be generated daily. The calibration method is saved and used to quantify all samples.

8. Sample Collection, Handling and Preservation

8.1 Grab samples are collected in pre-cleaned amber glass containers with Teflon-lined caps. Field blanks are needed to follow conventional sampling practices. Samples are collected and refrigerated. Automatic sampling equipment must be free of Tygon tubing and other potential sources of contamination.

8.2 All samples are iced or refrigerated at 4°C (± 2°C) from the time of collection until analysis.

8.3 At the laboratory, samples are stored in the refrigerator at 4°C or less until requested for analysis. The samples should be analyzed within 7 days of collection or as soon as possible. After injection in the LC/MS, the vial septa are replaced and the vials are stored in a refrigerator. The sample integrity due to decomposition of the target analytes is not addressed in this SOP and further sample collection and preservation studies need to be

undertaken.

8.4 Conventional laboratory practices involving chain of custody, field sampling, sampling protocols, preservation and holding times are referenced from CRL SOPs GEN004 and GEN013.

9. Analytical Procedure

9.1 Liquid Chromatography/Mass Spectrometry

9.1.1 The following are the liquid chromatographic conditions:

LC Chromatographic Column: (SIELC -Primesep SB™ 5 µm, 100 Å particle, 150 mm x 2.1 mm or equivalent).

Injections of all standards and samples are made at a 50 µL volume and are composed of water. Analyze the calibration curve and all samples in a high to low concentration regiment so carry-over is less of a concern in case the LC cleaning cycle does not take care of the system between injections adequately. The first sample analyzed after the curve is a blank to insure there is no carry-over. The gradient conditions for the liquid chromatographic are shown in Table 6.

All samples and blanks are filtered through a Millex HV syringe driven filter unit PVDF with 0.45 µm pore size to remove particulates in the water samples. The syringes must be rinsed to full volume 3 times with 50/50 acetonitrile/water between all field samples, QC samples, blanks and standards. Calibration standards are not filtered through the syringe driven filter units since no particulates are present.

9.1.2 The following are the mass spectrometer conditions:
Variable parameters depending on analyte are shown in Table 7.

The instrument is set in the Electrospray (+) positive source setting.
Capillary Voltage: 3.5 kV
Cone: Variable depending on analyte (Table 7)
Extractor: 2 Volts
RF Lens: 0.2 Volts
Source Temperature: 120 °C
Desolvation Temperature: 300 °C
Desolvation Gas Flow: 500 L/hr
Cone Gas Flow: 25 L/hr
Low Mass Resolution 1: 14.5
High Mass Resolution 1: 14.5
Ion Energy 1: 0.5
Entrance Energy: -1
Collision Energy: Variable depending on analyte (Table 7)
Exit Energy: 2
Low Mass Resolution 2: 15

High Mass resolution 2: 15
Ion Energy 2: 0.5
Multiplier: 650
Gas Cell Pirani Gauge: 3.3 x 10^{-3} Torr
Inter-Channel Delay : 0.02 seconds
Inter-Scan Delay : 0.1 seconds
Repeats: 1
Span: 0 Daltons
Dwell: 0.1 Seconds

9.1.3 If the absolute amount of a target compound exceeds the working range of the LC/MS system (see Level 7 in Table 3), the prepared sample is diluted with reagent water and re-analyzed.

9.1.4 If there are two or more analyses for a particular fraction due to sample dilution, the analyst must determine which is the best to report on the sample summary results sheet.

9.1.5 All qualitative and quantitative measurements are performed as described in Section 9.2. When not being analyzed, samples are stored in the refrigerator at 4°C or less and protected from light in screw cap top vials equipped with Teflon-lined septa.

9.2 Qualitative and Quantitative Analysis

9.2.1 An external calibration is used monitoring the SRM transitions of each analyte. Quanlynx™ Software is utilized to conduct the quantitation of the target analytes and surrogates. The SRM transitions of each analyte are used for quantitation and confirmation. This gives us confirmation by isolating the parent ion, fragmenting it to the daughter ion, and also relating it to the retention time in the calibration standard.

9.2.2 The Quanlynx™ manual should be consulted in order to use the software correctly. The quantitation method is set as an external calibration using the peak areas in ppt or ppb units as long as the analyst is consistent.

9.2.3 If the polynomial type is linear excluding the point of origin and a fit weighting of 1/X in order to give more weighting to the lower concentrations. The retention time window of the SRM transitions must be within 5% of the retention time of the analyte in the level 4 calibration standard. If this is not true the calibration curve needs to be re-analyzed to see if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample the analyte is referred to as an unknown. The coefficient of the determination, r^2, should be > 0.98 for each analyte. If one of the calibration standards, other than the high or low, causes the curve to be <0.98 this point must be re-injected or a new calibration curve must be made. If the low or high point is excluded, a five point curve is acceptable but the calibration range and reporting limits must be modified to reflect this change.

9.2.4 If the polynomial type is quadratic, the point of origin is excluded and a fit weighting of 1/X in order to give more weighting to the lower concentrations. The retention time window of the SRM transitions must be within 5% of the retention time of the analyte in the level 4 calibration standard. If this is not true the calibration curve needs to be re-analyzed to see if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample the analyte is referred to as an unknown. The coefficient of the determination, r^2, should be > 0.99 for each analyte. If one of the calibration standards, other than the high or low, causes the curve to be <0.99 this point must be re-injected or a new calibration curve must be made. If the low or high point is excluded, a six point curve is acceptable using a quadratic fit. An initial 7 point curve over the calibration range is suggested in the event the low or high point must be excluded to obtain a coefficient of the determination > 0.99. In this event, the calibration range and reporting limits must be modified to reflect this change.

10. Waste Handling and Pollution prevention

10.1 A green tag identifying the contents of the carboy is placed on the waste container. Remember to always attach the appropriate chemical waste label and date the beginning of collection before using the container.

10.2 Additional information on waste reduction is found in the laboratory safety facility plan.

11. Calculations and Documentation

11.1. Computer programs used for analysis of data include Masslynx™ with Quanlynx™ software.

11.2. Clarification of where professional judgment is used in the data reduction and verification process. CRL SOP GEN014 is followed for manual integration procedures.

11.3. All data packages are verified by a qualified analyst. The qualified analyst signs off on narrative and checklist. The data review is done using CRL SOP GEN010.

11.4. All QA/QC data and final results are reported to the customer.

11.5. Electronic data storage reference All data is archived to R5CRL/GCMS/Analysts Name/work order #.

12. Routine Analysis

12.1 The concentration in the water sample is calculated using the 1/X weighted calibration curve determined by the equation that fits the calibration curve.

The water sample results are reported in micrograms per liter (µg/L). These results are

reported without any corrections for recovery data. All QC data obtained are included in the CRL data packages.

12.2 Analytical Sequence

12.2.1 Prepare a sequence that includes all QC samples and field samples. The first sample to be analyzed is a 50 µL injection of a blank sample.

12.2.2 The calibration standards, Levels 7 through 1 are analyzed next. Verify that all analytes have been properly identified and quantified. Using software programs, manually integrate as necessary. Print quantitation reports for the calibration standards.

12.2.3 Update the calibration file and print a calibration report. Review the report for calibration outliers and make area corrections. If corrections have been made, update the calibration file and regenerate a calibration report. Alternatively, re-analyze "nonconforming" calibration level(s) and repeat the above procedures.

12.2.4 If the initial calibration data are acceptable samples are analyzed during the 24-hour period ending with a midpoint calibration standard. The ending midpoint calibration standard must have each analyte concentration within 30% of the calculated true concentration or the affected analytes from that run must be qualified estimated or re-analyzed with passing criteria to remove the qualification. This insures that there is minimal instrument drift and/or MS interface interferences which can cause matrix enhancement or suppression.

12.2.5 The next samples to be analyzed should be in the following recommended sequence: Lab Blank, LCS, diluted samples, samples and MS/MSD followed by a midpoint calibration standard to check instrument stability over the analysis period.

12.2.6 Archive all the raw data promptly to the R5CRL server.

12.2.7 Generate quantitation reports for all samples following data system specifications and the Chromatographic Peak Interpretation SOP (QMP Appendix 2). Generate the final data. Copies of the original and final sequence listings should be included in the data packages as well as in the Instrument Sample Log binders. If manual integration was used the Quanlynx Audit Report is accessible showing the details how, when and why the peak was changed. The entire quantitation method is saved and archived.

12.2.8 Review the quantitation reports for all samples making sure all surrogate and target compounds have been properly quantitated. Check for integration errors.

12.2.9 Review the quantitation reports for all samples. Delete any false positive method specific parameters results that are less than the method detection limits listed in Table 1.

12.2.10 Create sample header and miscellaneous information files for all samples in the analytical sequence.

12.2.11 Generate the report forms using Quanlynx™ software.

12.2.12 Be sure the blank sample data have been properly reviewed. Then generate a QC form listing field samples prepared with the associated lab blank sample.

12.2.13 Verify that all spike compounds were present on the MS and MSD and LCS sample quantitation reports. Investigate any gross deviations in spike concentrations from expected values.

12.2.14 Generate analysis data sheets for blank, and field samples. Review the final results and upload the applicable data (sample results, surrogate spike compound recovery data and matrix precision and accuracy data) into CRL LIMS, when available.

12.2.15 On a daily basis archive all the processed data files to their designated archiving location in the R5CRL server (see the CRL SOP # GEN002).

12.3 Data Package Assembly

12.3.1 Create a calibration deliverables package with the following: copies of sequence files, initial and continuing calibration reports, and quantitation reports for calibration standards.

12.3.2 Create a QC deliverables package with applicable QC Forms.

12.3.3 Create a sample deliverables package with the following for each sample: sample data sheet, sample quantitation report. Make sure all method specific parameter peaks are correctly labeled on the chromatogram.

12.4 Data Package Quality Control

12.4.1 Analysts are primarily responsible for performing data evaluation and verification tasks. See CRL SOP (GEN010) on data review, validation and qualification criteria. For data qualification purposes, apply the QC criteria specified in this SOP.

12.4.2 All data packages must be approved and signed by a qualified Analyst and a secondary reviewer prior to release.

12.5 Data Reduction and LIMS Entry and Reporting (Automatic upload is still not available for LC/MS analysis)

12.5.1 All LIMS data entry is based on first creating a bench sheet describing the sample preparation. This bench sheet describes the samples prepared, and the quality control samples. The analyst must make certain that the preparation date in LIMS matches the actual preparation date. By convention, if the sample preparation proceeds overnight, the date started is used for the LIMS preparation

date.

12.5.2 When the data are ready to be entered, the bench sheet is called up into the Data Entry/Review module. All analyses or selected analyses can be included. If only a few analyses are to be entered, data may be entered manually. Otherwise, it may be more practical to use Data Tool.

12.5.2.1 When performing manual data entry, enter the results in the column **Result**. For each result, enter the date of analysis in the column **Analyzed**. This column has a calendar feature as do other date fields in LIMS. If dilutions were necessary for the analysis, enter the dilution in the column **Dilution**. The sample result should be the one measured and not corrected for the dilution factor. Verify that the correct initials are present in the Analyst field and the instrument field.

12.5.2.2 If all data are entered, click the **Save** button on the top row. After saving, proceed to the Review page by clicking **Query** on the second row. Verify that all conversions to reporting units and dilutions have been calculated correctly. Verify that reporting limits have been correctly applied. Flags may be added at this stage, following the guidance given in the GC/MS Data Review SOPs. Before review by the secondary reviewer, the data may be locked, and the status should be updated to analyzed.

12.5.2.3 If Data Tool is to be used, once the batch is called up in Data Entry/Review, click **Export** to create an Excel file in the User Directory. Name this file in such a manner that it can be easily associated with that analysis.

12.5.2.4 A file created by the instrument (a .txt file from Quanlynx™) with the instrument readings for each sample. If multiple measurements for a given sample are present, such as for dilutions, the .txt file can be loaded into a text editor, such as Word Pad, and the sample IDs for the sample readings that are not to be used can be altered so that Data Tool does not recognize them. This altered file must be saved as a .txt file for Data Tool to use it. There is no need to remove the analytical spikes, because the software recognizes them as such.

12.5.2.5 Once in Data Tool, click **Browse** for the Element Data Entry Table, and call up the .xls file created earlier. If unneeded sample entries remain in the lower left-hand box, click **Clear**. Double-click on the desired .txt file and either Auto Select or highlight individual samples and click **Include**.

12.5.2.6 Once the field samples and quality control samples are selected, click **Done,** and it will return to the main Data Tool page. Click **Merge Files**. If either Unmatched Analytes or Unmatched Units appear in red, repair the cross table with the assistance, if necessary, of the GC/MS Group Leader. Verify that the results in Initial Result are correct, and click **Save**, which will create an Excel file. Name this one differently from the name chosen previously and click **Done**.

12.5.2.7 Return to the Data Entry/Review module and click **Open**, using the .xls file created in the paragraph above. Verify that all items are correct as in the manual data entry in 12.5.2.1 and click **Save**. Query the data and proceed as in 12.5.2.2.

12.6 LIMS Report Generation

12.6.1 Once all LC/MS data is entered with the status of Analyzed, prepare a draft report. In LIMS, select Project Management, Reports. Choose the work order and the analyses, and select the report. For LC/MS analyses, the LIMS analyses are all multiple element analyses as of this writing. The report chosen will typically be C_Analysis.rpt or C_Sample.rpt. If QC is to be omitted from the report, choose Modified Draft, unchecking the quality control samples that were not analyzed. This draft report need not be signed. It is only for the purpose of review.

After the peer reviewer has updated the status of the LIMS entries to Reviewed, the final report may be generated. The mode of generation of the report is the same as above, and either the Final Report or Modified Final Report is chosen. All pages of the report and the transmittal form must be signed and dated.

13. Quality Control

Refer to Region 5-Chicago Regional Laboratory SOP GEN010 for a detailed QA/QC protocol.

A-Initial demonstration, which include precision and accuracy (P&A) study and LOD:

13.1 An initial demonstration of the laboratory capability to generate data of acceptable quality must be done. A precision and accuracy (P&A, as shown in Table 2 and Section 17 Attachments) study must be performed whenever a major modification is made to this method.

13.1.1 For a precision and accuracy (P&A) study, a check standard containing the thiodiglycol and 3.3'-thiodipropanol near or below the midpoint concentration in the calibration table must be analyzed, a minimum of 4 replicates. The check samples are then analyzed according to the method beginning in Sections 9 and 10.

13.1.2 The average percent recovery (X), and the standard deviation (σ) of the recoveries is calculated for each analyte. Establish the QC confidence limits at 95.4% (2σ).

13.2 Limit of Detection Limit Test: A mixed stock solution of the target analytes will be serially diluted to achieve standards of successively lower concentrations. These standards will be diluted to a concentration which is below the ORD-NHSRC risk criteria. If the risk criteria level is insufficient for detection, ORD-NHSRC will be consulted. The Limit of Detection (LOD) will be determined for each of these analytes. The LOD will be determined by the signal to noise ratio at a concentration < one third of the lowest point of the calibration curve. An environmental water matrix will be used to

conduct the initial signal to noise determination. Adequate signal to noise at this level should be greater than 3:1. Since the LOD is dependent upon matrix, instrument type and instrument maintenance, LOD will be provided in the procedure as guidance only. Data for LODs are shown in Table 1 and Section 17 Attachments.

B- On going QC:

13.3 LCS/LCSD Spikes

As part of the Region 5 CRL QC program, spike accuracy for a clean matrix (in this case reagent water) is monitored and updated regularly. LCS/LCSD spikes should be prepared at a frequency of at least one pair for every 20 samples. Records are maintained of the reagent water target compound spike analyses and the average percent recovery (X) and the standard deviation of the percent recovery (σ) are calculated. This procedure maintains a 95.4% confidence interval from $X \pm 2\sigma$ to use as upper and lower control limits for evaluation of spiked target compound recovery.

13.4 Surrogates:

As part of the Region 5 CRL QC program, all samples are spiked with surrogate standard spiking solution as described in Section 7. An average percent recovery of the surrogate compound and the standard deviation of the percent recovery is calculated and updated regularly. This procedure maintains a 95.4% confidence interval from $X \pm 2\sigma$ control limits for surrogate compounds.

13.5 Reagent Blank:

A reagent blank is prepared each day, with analyte free water with a frequency of at least one for every 20 samples to investigate for system/laboratory contamination.

13.6 MS/MSD:

As part of the Region 5 CRL QC program, target compound spike accuracy in the sample matrix is monitored and updated regularly. Records are maintained of spiked matrix analyses and the average percent recovery (X) and corresponding standard deviation (σ) are calculated. This procedure maintains a 95.4% confidence interval of $X \pm 2\sigma$ to use as upper and lower control limits for evaluation of spiked compound recovery in the sample matrix. When required, a matrix spike and matrix spike duplicate (MS/MSD - section 7.2) are prepared with a frequency of at least one MS/MSD pair for every 5 samples to investigate for matrix interferences. If the laboratory has not received MS/MSD samples for site specific precision and accuracy (P&A) data, the laboratory will evaluate the site data quality based on the Lab Control Sample (LCS - section 7.2) data. If there is enough sample in the site samples to conduct MS/MSD and duplicate analysis that will be used for site specific precision and accuracy (P&A) data.

14. Troubleshooting Guide and Preventive Maintenance

There are many reasons an instrument or analysis may fail, a few are noted here and may be updated periodically.

14.1 Symptom: Inadequate Abundance or Sensitivity

Probable Causes:

14.1.1 Dirty or contaminated ion source, electron multiplier, or quadrupole rod surfaces.

14.1.2 Potentials of ion source elements at wrong values due to open or short circuits.

14.1.3 Faulty ion source electronics, detector electronics or power supply.

14.2 Symptom: Improper Isotope Ratios

Probable Causes:

14.2.1 High "background" levels of undesired substances (Contamination from earlier sample injections) which contribute additional abundance at the isotope mass. Bake out the ion source assembly and condition the column.

14.2.2 Resolution of adjacent masses set improperly: higher than normal ratios due to poor resolution (peaks too wide) or lower ratios due to over resolution (narrow peaks).

14.3 Symptom: Low Overall Sensitivity

Probable Causes:

14.3.1 Dirty or contaminated ion source, electron multiplier or quadrupole rod surfaces.

14.3.2 Electron multiplier with low gain.

14.4 Symptom: Poor Reproducibility

Probable Causes:

14.4.1 Loose or intermittent connection either to a printed circuit or to one or more ion source or quadrupole elements inside the analyzer assembly.

14.5 Symptom: High Background

Probable Causes:

14.5.1 Dirty or contaminated ion source, electron multiplier or quadrupole rod surfaces. Bake out the source.

14.5.2 "Yesterday's" Samples - There is the possibility that some previously injected sample can still be present in the vacuum system long after it was thought to be evacuated. This phenomenon depends on sample volatility, temperature, etc.

14.5.3 Contamination in a recently cleaned vacuum system - After any venting of a vacuum system for maintenance, there is the potential for introducing new substances into the vacuum system. Some substances are normal and can be

pumped out, while others require more cleaning or baking.

14.5.3.1 Solvents used in the cleaning process: These will be present for a while but should be pumped out as heat is applied to the vacuum system.

14.5.3.2 Water absorbed on the metal surfaces while vented. This will pump out with heat.

14.5.3.3 "Fingerprints" - Heavy organic substances from inadequate clean room procedures may not be pumped out and may require source cleaning.

14.6 Symptom: Mass Spectrometer Does Not Respond

Probable Cause:

14.6.1 The mass spectrometer electronics are not on - Check the switch.

14.6.2 Secondary fuse blown - Check the secondary fuses on the rear of the mass spectrometer and replace the faulty fuse or fuses.

14.6.3 Board failure

15. References

Code of Federal Regulations, 40 CFR Part 136, Appendix B.

Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants: May 1977, Revised April 1977; U.S. Environmental Protection Agency. Environmental Monitoring Support Laboratory, Cincinnati, Ohio 45268. Available from Effluent Guidelines Division, Washington, DC 20160.

Standard Practices for Preparation of Sample Containers and for Preservation of Organic Constituents, American Society for Testing and Materials, Philadelphia. ASTM Annual Book of Standards, Part 31, D3694-78.

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OSHA Safety and Health Standards, General Industry, (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206 (Revised, January 1976).

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Methods 330.4 (Titrimetric, DPD-FAS) and 330.5 (Spectrophotometric, DPR) for Chlorine, Total Residual, Methods for Chemical Analysis of Water and Wastes, U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio 45268, March 1979. EPA-600/4-79-020

Eichelberger, J.W.; Harris, L.E.; Budde, W.L. "Reference Compound to Calibrate Ion Abundance Measurement in Gas Chromatography/Mass Spectrometry" *Analytical Chemistry* **1975,** *47*, 995.

McNair, N.M.; Bonelli, E.J. *Basic Chromatography*;, Consolidated Printing: Berkeley, CA, 1969; p. 52.

Burke, J.A. "Gas Chromatography for Pesticide Residue Analysis; Some Practical Aspects", *J. Assoc. Off. Anal. Chem.* **1965,** *48*, 1037.

Olynyk, P.; Budde, W.L.; Eichelberger, J.W. "Method Detection Limit for Methods 624 and 625" Unpublished report, October 1980.

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Extraction of Soil Samples Using Dionex ASE 200, USEPA Region 9 Draft SOP, January 11, 1999.

16. Appendices

Tables and Validation Data

Item	Title	Number of Pages	Revision Number	Date Revised
Table 1	Method Parameters	1	0	09/2007
Table 2	Quality Control Acceptance Criteria	1	0	09/2007
Table 3	Concentration of Calibration Standards	1	0	09/2007
Table 4	Preparation of Calibration Standards	1	0	09/2007
Table 5	Retention Times and MRM Ions	1	0	09/2007
Table 6	Gradient Conditions for Liquid Chromatography	1	0	09/2007
Table 7	Variable Mass Spectrometer Parameters Depending on Analyte	1	0	09/2007

TABLE 1.

MS015- Method Parameters

PARAMETER	CAS#	LOD (µg/L)*, (Signal/Noise Ratio)	Reporting Limit** (µg/L)
Thiodiglycol	111-48-8	50, (27)	250
3,3'-Thiodipropanol (Surrogate)	10595-09-2	50, (20)	250

*Limit of Detection Limits were done in Chicago River Water and will be updated regularly. Last LOD Study- September 2007.

**Reporting Limit concentrations are calculated from Table 3 Level 2 concentrations assuming a 50 µL injection of the lowest level calibration standard.

TABLE 2.

Quality Control Acceptance Criteria

PARAMETER	% Recovery* LCS/LCSD Reagent Water	% Recovery* MS/MSD Environmental Water
Thiodiglycol	80-140	74-134
3,3'-Thiodipropanol (Surrogate)	82-142	93-153

Currently, LCS spike recovery limits are used to assess site sample data quality. Matrix Spike, Matrix Spike Duplicate and Lab Control Sample spike recovery limits will be revised annually or upon collection of 20 QC data points, whichever is sooner.

*The quality control acceptance criteria shall be updated regularly. **Preliminary Acceptance Criteria are set at ±30% of the average recovery based on reagent and river water as shown in Section 17 Attachments.**

LAST UPDATE September, 2007

TABLE 3.

Concentrations of Calibration Standards (µg/L).

Target/Surrogate	Level 1*	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
Thidiglycol	100	250	500	1,000	2,500	5,000	10,000
3,3'-Thiodipropanol	100	250	500	1,000	2,500	5,000	10,000

***Level 1 is below the reporting limit and above the limit of detection, if your instrument is capable obtaining good signal/noise ratio at this level it may be added and your reporting limit adjusted to 100 ppb.**

TABLE 4.

Preparation of Calibration Standards.

Solution	LV1	LV2	LV3	LV4	LV5	LV6	LV 7
A	10 µL	25 µL	50 µL	100 µL	250 µL	500 µL	1000 µL
B	990 µL	975 µL	950 µL	900 µL	750 µL	500 µL	0 µL

Solution A: Stock solution prepared in Section 7.3 and at Table 3 concentrations.
Solution B: Water.

TABLE 5.

Retention Time (RT) and SRM Ions

Parameter	**RT (minutes)**	**SRM**
Thiodiglycol	**2.8**	**123.1 >104.9**
3,3'-Thiodipropanol	**6.3**	**151.2>133.1**

TABLE 6.

Gradient Conditions for Liquid Chromatography.

Time (minutes)	Flow (µL/minute)	Percent Acetonitrile	Percent Water	Percent 500 mmolar Ammonium Formate/2% Formic Acid in Water
0	300	0	95	5
2.5	300	0	95	5
6	300	90	5	5
10	300	90	5	5
12	300	0	95	5
16	300	0	95	5

*Column Temperature at 30°C, Sample compartment at 15°C.
Equilibration time- 2 minutes.

TABLE 7.

Variable Mass Spectrometer Parameters Depending on Analyte.

Analyte	SRM	Cone (Volts)	Collision Energy (eV)
Thiodiglycol	123.1>104.9	18	5
3,3'-thiodipropanol	151.2>133.1	19	8

TABLE 8.

Uncertainty Determination

To Be Determined

17. Attachments

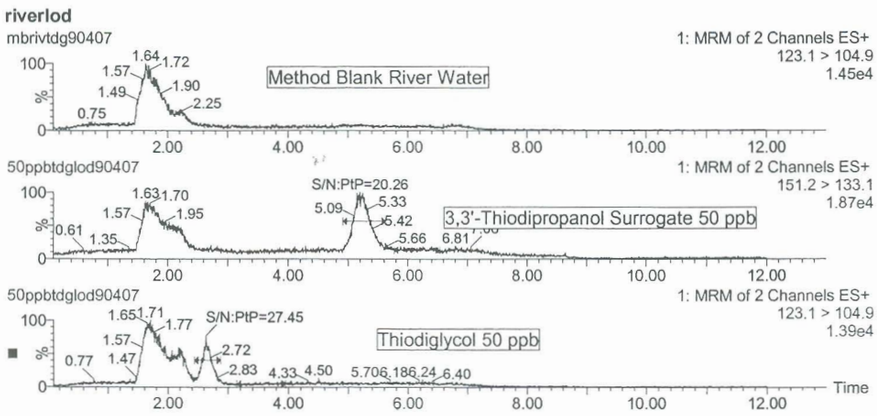
17.1 Limit of Detection Study

17.2 Precision and Accuracy

17.3 Representative Calibration Curves

17.1 Limit of Detection Study

The Limit of Detection study was done in Chicago River Water.



17.2 Precision and Accuracy

Precision and Accuracy Data for Thiodiglycols
Calibration Standard in Water
All Target Compounds spiked at 2.5 ppm
3,3'-thiodipropanol Surrogate spiked at 2.5 ppm

3,3'-thiodipropanol (Surrogate) 151.2>133.1	Spike (ppm)	Recovered (ppm)	Percent Recovery	
Method Blank Reagent Water 8/9/07	2.5	2.8	112.4	
P&A 1 Reagent Water 8/9/07	2.5	2.8	112.8	
P&A 2 Reagent Water 8/9/07	2.5	2.7	109.6	
P&A 3 Reagent Water 8/9/07	2.5	2.8	112.4	
P&A 4 Reagent Water 8/9/07	2.5	2.9	114.4	
P&A 5 Reagent Water 8/9/07	2.5	2.9	116.0	
		2.8	112.9	Average Recovery
		0.1	2.2	Standard Deviation

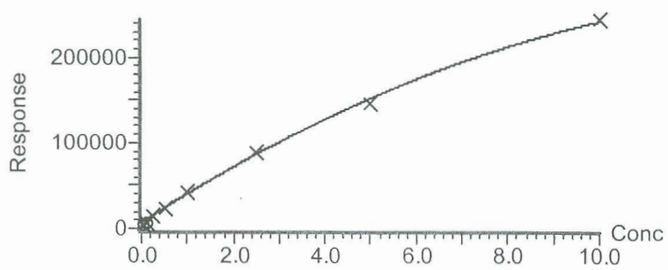
3,3'-thiodipropanol (Surrogate) 151.2>133.1	Spike (ppm)	Recovered (ppm)	Percent Recovery	
Method Blank Chicago River Water 8/9/07	2.5	2.8	110.8	
Method Blank 2 Chicago River Water 8/9/07	2.5	2.9	115.2	
P&A 1 Chicago River Water 8/9/07	2.5	3.2	126.4	
P&A 2 Chicago River Water 8/9/07	2.5	3.2	128.0	
P&A 3 Chicago River Water 8/9/07	2.5	3.2	126.4	
P&A 4 Chicago River Water 8/9/07	2.5	3.2	127.6	
P&A 5 Chicago River Water 8/9/07	2.5	3.2	128.8	
		3.1	123.3	Average Recovery
		0.2	7.2	Standard Deviation

Thiodiglycol 123.1>104.9	Spike (ppm)	Recovered (ppm)	Percent Recovery	
P&A 1 Reagent Water 8/9/07	2.5	2.7	109.6	
P&A 2 Reagent Water 8/9/07	2.5	2.8	110.0	
P&A 3 Reagent Water 8/9/07	2.5	2.7	109.2	
P&A 4 Reagent Water 8/9/07	2.5	2.8	112.0	
P&A 5 Reagent Water 8/9/07	2.5	2.8	112.0	
		2.8	110.6	Average Recovery
		0.0	1.3	Standard Deviation

Thiodiglycol 123.1>104.9	Spike (ppm)	Recovered (ppm)	Percent Recovery	
P&A 1 Chicago River Water 8/9/07	2.5	2.6	102.0	
P&A 2 Chicago River Water 8/9/07	2.5	2.6	105.6	
P&A 3 Chicago River Water 8/9/07	2.5	2.6	105.2	
P&A 4 Chicago River Water 8/9/07	2.5	2.6	103.6	
P&A 5 Chicago River Water 8/9/07	2.5	2.6	105.2	
		2.6	104.3	Average Recovery
		0.0	1.5	Standard Deviation

17.3 Representative Calibration Curves

Compound name: Thiodiglycol
Coefficient of Determination: R^2 = 0.998078
Calibration curve: -1138.28 * x^2 + 34850.7 * x + 6752.52
Response type: External Std, Area
Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x, Axis trans: Nc



Compound name: 3,3'-thiodipropanol
Coefficient of Determination: R^2 = 0.998845
Calibration curve: -7004.91 * x^2 + 195032 * x + 18591.5
Response type: External Std, Area
Curve type: 2nd Order, Origin: Exclude, Weighting: 1/x, Axis trans: Nc